PROPOSAL FOR TOPIC APPROVAL TO BE SUBMITTED TO THE
UNIVERSITY OF MUMBAI FOR THE DEGREE OF
Ph.D. (Tech) IN THE SUBJECT OF PHARMACEUTICAL CHEMISTRY

Title of the Topic	:	Identification and Optimization of Lead molecules from Natural Origin for PCOS
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Introduction:

Polycystic Ovarian Syndrome (PCOS) is a serious heterogeneous endocrine disorder in women in which ovaries become enlarged with many cysts termed as undeveloped follicle formation. PCOS is also termed as Stein-Leventhal Syndrome. PCOS is characterized by irregular periods enhancing androgen secretion, anovulation, amenorrhea, infertility, hair thinning, acne, obesity, infertility, insulin resistance, hypertension, dyslipidemia, pain in pelvic region.[1] It is associated with psychological impairment like depression, derangements, increased level of Luteinizing Hormone and decreased Follicle Stimulating Hormone, hirsutism. The excess of androgen levels are the main cause of PCOS which results from disturbances in GnRH pulses that give rise to increased level of LH/FSH ratio. According to the National Institute of Health Office of Disease Prevention, PCOS affects approximately 5 million women of child bearing age. Research suggests that 5-10% of females at 18 to 44 years of age are affected by PCOS making it the most common endocrine abnormality among women of reproductive age.[8]

Insulin resistance resulting in hyperinsulinemia was first identified by Burghen (1980) as playing a key role in the pathogenesis of PCOS. Dunaif (1997) developed this finding to show that insulin resistance has a variety of circulatory, metabolic, hormonal and inflammatory consequences on the body. Hyperinsulinemia is understood to be the pathogenic mechanism involved in the development of hyperandrogenism. High levels of insulin stimulate the ovaries and adrenals to over produce androgens. This results in the absence of ovulation and consequent hyperandrogenism. Hyperinsulinemia decreases the hepatic production of the sex hormone binding globulin, which in turn further increases the amounts of circulating androgens. Insulin resistance is present in skeletal muscle and adipose tissue. With PCOS, there are defects in both insulin action and secretion and the molecular mechanism differ from those found in obesity and NIDDM (Dunaif 1997).[11] The GnRH frequency in the hypothalamus is higher than normal. This results in increased levels of LH relative to FSH. This hormonal imbalance further results in increased levels of androgens.[6]

Reducing body weight of anovulatory obese women decreases insulin resistance, testosterone concentration and restores ovulation. [7]

The most common general causes identified of PCOS are Genetic predisposition, Strong stimulation in adrenals in childhood, Raised insulin level, Contraceptive pills, Hormonal imbalance and Stress.[3] Histological features of PCOS include thickened capsule of size > 100μ, Hyperplasia, Ovarian hypertrophy, scarcity of corpus luteum. In severe cases, it may become associated with dysfunctional uterine bleeding, Type II Diabetes, endometrial cancer, high cholesterol and cardiovascular disease.[4]

The Allopathic remedies available for PCOS are Nafarelin (a specific gonadotropin-releasing-hormone agonist), Triglitazone, Tamoxifen, Clomiphen (partially selective estrogen receptor modulator), Metformin, Spironolactone, Estrogen, Progestins, Eflornithine and Laproscopy.[5]

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Natural alternatives available for treatment of PCOS are adequate intake of Calcium, Vitamin D, and Magnesium (low levels of Mg associated with Diabetes). It also includes increased intake of Chromium & Turmeric (essential for insulin and blood glucose level), omega-3 supplements (decreases Androgen level in women with PCOS), rich fibre diet, Anti-inflammatory foods like olive oil, tree nuts, tomatoes, leafy green vegetables, fatty fish like Mackerel and Tuna, totally cut off the coffee as it causes changes in estrogen level. The synergistic effect among different plant constituents can be possible in treatment of PCOS and for that Pharmacological fractionation is one of the method to check and confirm the plant resources.

Rational:

PCOS is a very common and severe syndrome seen that affects about one in 15 women worldwide. Currently many pharmacological and allopathic treatments are available to cure PCOS like Antiandrogens, Clomiphen Citrate, insulin sensitizers and hormonal therapies. It seems that only symptomatic treatment is available for PCOS treatment. Though, these therapies are effective but they cost tremendously and unfortunately may cause various side effects. The herbal medicines work with the body whereas the conventional therapies modify the physical condition. The successful treatment of infertility is usually possible in the majority of patients with PCOS by using natural, non-invasive and non-chemical remedies.

Some natural molecules are available for patients suffering from PCOS like α-Thujone, Berberine, Curcumin. There are evidences in literature of testing these remedies on patients with PCOS and insulin resistance evaluating parameters such as blood lipid level, Glucose level, Ovulation, effect on corpus luteum, implantation and obstetrics complications, ovarian histomorphology.

Coumarins are widely seen in nature and they make in the list of remedies for PCOS. Also, based on literature review, Coumarin moiety is selected as a lead for experimentation and studies as it's easy to extract from natural origin, easy to synthesize and modify although no specific mechanism has been reported of it to treat PCOS. The natural Coumarins can be easily modified to yield some good derivatives and can be characterised, standardised and analysed by animal study.[12, 13]

Aim and Objective: The aim of present work is

1. To extract the Coumarin from natural origin,
2. To identify their roles in treatment of PCOS
3. To synthesize them chemically and modify them to get some good derivatives. Synthesis of these Coumarin molecules can be planned. Different methods can be developed and used for the same. Certain methods available for synthesis of Coumarins are enlisted under materials and methods
4. To have an computer aided insight to the scientific problem by using suitable techniques
5. To test the hypothesis by using animal models and pharmacological testing there on.

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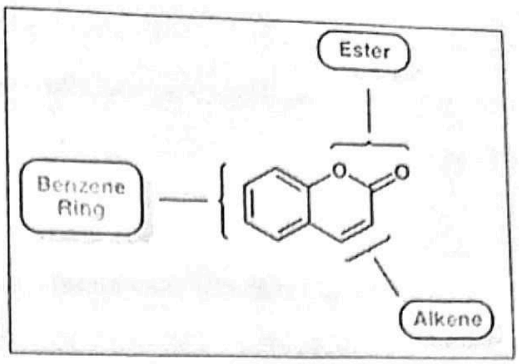

Figure 1: General Scaffold of Coumarin

Plan of Work

Coumarin is an organic compound that has two six-membered rings fused together, with one of the rings being a benzene ring and the other containing alkene functionality and an ester functional group. Coumarins play an important role in both natural systems like plants and also in medicinal applications as drug molecules. Coumarin actually belongs to a broader class of compounds called the benzopyrones.

Materials and Methods:

The herbs identified in the literature are *Menthapiperita, Menthaspicata, Cinnamon cassia, Tribulusterrestris, Glycyrrhiza species, Origanummajorana, Tinospora cordifolia, Asparagus racemosus, Trigonella foenumgraceum, Vitexnegundo, Foeniculum vulgare, Cyanodon dactylon, strawberries, tonka beans, apricots, cherries, sweet clove, lavender, Cimcifuga racemosa, Anethum graveolens (Shatapushpa), Aegle marmelos (Bael)* [2, 9]

1) Certain herbs can be used to extract Coumarin from them by various extraction procedures.

Extract procedure and solvents: Water extract - To prepare root extract 10grams of prepared powder from selected plant is individually placed in 250 ml conical flask by adding 100 ml of distilled water to it such that plant powder soaked in it and shaken well. The solution then filtered with the help of filter paper and filtered centrifuge at 20 minute. Then the solutions were labelled Extract of the selected plant samples were taken and used for further phytochemical analysis. The water extract was kept in refrigerator when not in use. (b) Alcoholic extract- To prepare root extract 10grams of prepared powder from selected plant is individually placed in 250 ml conical flask by adding 100 ml of 95 percent alcohol to it such that plant powder soaked in it and shaken well. The solution then filtered with the help of filter paper and filtered centrifuge at 20 minute. Then the solutions were labelled Extract of the selected plant samples were taken and used for further phytochemical analysis. The alcoholic extract was kept in refrigerator when not in use.[10]

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2) The next step could be to identify the role of Coumarin in treatment of PCOS
3) Chemically, synthesis of Coumarin and their different derivatives can be planned, also modify them further to check their effectiveness. Certain synthetic reactions are listed below[11]

i) Pechmann Condensation: Reaction of phenols with β-keto esters
ii) Knoevenagel Condensation Reaction: Efficient green one pot synthesis for 3-substituted Coumarins of reaction of o-hydroxybenzaldehyde with 1, 3-dicarbonyl compounds
iii) Baylis Hillman Reaction: Reaction of 2-hydroxy Benzaldehyde with Methyl Acrylate
iv) Kostanecki Reaction: 4-Aryl Coumarins can be synthesized by employing reaction between 2-hydroxy Benzophenone and Acetic Anhydride
v) Michael addition can be done for the synthesis of 3-Aroyl Coumarins
vi) Wittig Reaction
vii) 4- Carboxymethyl Coumarins can be synthesized
viii) Palladium catalyzed reaction between substituted phenol and propiolic acid
ix) Synthesis of 3, 4-disubstituted Coumarins
x) Synthesis of 3-Alkyl Coumarins by reaction of 2-hydroxy Benzaldehyde and α,β- unsaturated aldehyde
xi) Perkin Condensation

Like this different Coumarin derivatives can be synthesized. Figure 1 indicates the various positions that can be explored in Coumarin core structures, however it will depend on active component (s) identified during the endeavour. If Coumarins doesn't work out, we could make it for Flavones as they are natural molecules, easy to synthesize in the laboratory and used in the treatment of PCOS.

4) Computer aided drug design can be employed for identifying the lead compound
5) Pharmacological model and drug testing: The synthesized lead compound can be tested on PCOS induced models to check its effectiveness.

Expected results/Outcomes:

1. Utility of Coumarin in PCOS treatment will be explored
2. SAR / Pharmacophore for the same will be established
3. Testing the hypothesis using pharmacological evaluation

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